ABSTRACT

The present invention provides a fast, simple and specific method for generating amplified messenger RNAs rather than antisense RNAs from limited intracellular RNAs. The principle of this RNA-polymerase chain reaction method relies upon the cycling steps of reverse transcription, denaturation, double-stranded cDNA synthesis and then in vitro transcription to bring up the amount of messenger RNAs to two thousand folds within one round of the above procedure. This method is primarily designed for differential screening of tissue-specific gene expressions in single cell level, cloning full-length sequences of unknown gene transcripts, generating probes for hybridization assays, synthesizing peptides in vitro, and preparing representative cDNAs for modern gene chip technology. In conjunction with a cell fixation and permeabilisation step, a complete full-length mRNA or cDNA library can be directly generated from few single cells without mRNA degradation.